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IL-10 and *IL-12B* gene polymorphisms in a multiethnic Malaysian population

S.S. Sam, B.T. Teoh and S. AbuBakar

Tropical Infectious Diseases Research and Education Center,
Department of Medical Microbiology, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

Corresponding author: S. AbuBakar
E-mail: sazaly@um.edu.my

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ABSTRACT. Inheritance of polymorphisms in the interleukin (IL)-10 promoter and IL-12B genes, which influence cytokine production and activities, may define the balance in T helper response in infection and autoimmune diseases. In the present study, we investigated the distribution of the *IL-10* promoter and *IL-12B* gene polymorphisms in a multiethnic Malaysian population. Overall, our findings suggest that the *IL-12B* and *IL-10* -592 genotypes were distributed homogenously across all major ethnic groups, including Malays, Chinese, and Indians, except for polymorphisms at *IL-10* -1082. At this gene locus, the ethnic Chinese showed a significantly lower allele frequency of -1082G (2.1%) compared to the Malay (12.2%) and Indian (15.3%) populations. Results for the *IL-12B* and *IL-10* gene polymorphisms were consistent with those reported for the Asian population, but markedly different from those of the African and Caucasian populations. Our findings suggest that there are specific genetic variations between different ethnic groups, which should be examined in all gene population-based association studies.

Key words: Infectious disease; Interleukin-10; Interleukin-12B; Ethnic; Malaysia; Polymorphisms

INTRODUCTION

Cytokines play a key role in regulating the T helper (Th) immune response. Inheritance of specific polymorphisms in the cytokine genes, including those located in promoter or coding regions, modifies gene transcriptional activities and cytokine production. Inter-individual variability in the cytokine profile may influence the immune response pattern against infections and in autoimmune diseases. Polymorphisms in the tumor necrosis factor alpha gene (*TNF-α*), for example, have been shown to be associated with clearance of hepatitis C virus infection and susceptibility to cerebral malaria (McGuire et al., 1994; Thio et al., 2004). Polymorphisms in the interleukin-12B (*IL-12B*) and *IL-10* genes may directly influence the Th and T regulatory balance (Miteva and Stanilova, 2008).

IL-12 is an immunoregulatory cytokine that upregulates the Th1-type immune response. Th1 response plays an important role in the battle against viral and intracellular bacterial infections, while an exaggerated Th1 response may contribute to the pathogenesis of autoimmune diseases. Several functional polymorphisms were identified within *IL-12B* and are associated with variations in gene expression and protein production levels; these include an insertion-deletion (CTCTAA/GC) in the promoter region and a *Taq*I single nucleotide polymorphism (SNP) in the 3'-untranslated region (UTR). Despite the effects of *IL-12B* polymorphisms, IL-12 secretion can be influenced by the level of IL-10 secretion (Peng et al., 2006). Within the *IL-10* promoter, 3 bi-allelic polymorphisms at positions -1082, -819, and -592 were reported to be associated with IL-10 production. Polymorphisms at these gene loci may directly influence the outcome of gene-disease association studies, particularly in a multiethnic population. In this study, we investigated the distribution of gene polymorphisms among the 3 major ethnic populations in Malaysia.

MATERIAL AND METHODS

The present study was approved by the University Malaya Medical Center Medical Ethics Committee (Ethics Committee/IRB reference number: 611.10). The study population included 278 randomly chosen samples of patients from the University Malaya Medical Center with suspected viral fever from 2006-2007 and 69 unrelated healthy volunteers. Written consent was obtained from healthy volunteers but not from the patients, as they were retrospectively enrolled in the study. Data collection and reporting were conducted anonymously and under the supervision of the University Malaya Medical Center medical records department. Patients' clotted blood samples were obtained from the Microbiology Laboratory Diagnostic Repository for genomic DNA extraction.

Genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen; Hilden, Germany) following the manufacturer protocol. Allele-specific primers (Mueller et al., 2004) were used to detect *IL-12B*pro (CTCTAA/GC) polymorphisms (rs17860508) (Table 1). The *IL-12B* 3'UTR *Taq*I polymorphism (rs3212227) and the 2 SNPs in the *IL-10* gene promoter at positions -592 (rs1800872) and -1082 (rs1800896) were genotyped using polymerase chain reaction coupled with restriction fragment length polymorphisms, as previously described (Edwards-Smith et al., 1999; Davoodi-Semiromi et al., 2002) (Table 1). The *IL-10* -819 SNP (rs1800871) was not genotyped as there is complete linkage disequilibrium between *IL-10* -819 and -592 SNPs. Genotyping results for the *IL-10* promoter SNPs in at least half of the samples was verified by nucleotide sequencing performed in 1 direction using the BigDye

Terminator v3.1 Cycle Sequencing Kit on a capillary DNA sequencer 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Table 1. Amplification primers for the detection of *IL-10* and *IL-12B* gene polymorphisms.

Gene polymorphisms	Genotyping method	Primer sequence (5'→3')	Restriction enzymes	References
IL10-592C/A	PCR-RFLP	FP-592: CCTAGGTCACAGTGACGTGG RP-592: GGTGAGCACTACCTGACTAGC	*Rsa*I	Edwards-Smith et al., 1999
IL10-1082G/A	PCR-RFLP	FP-1082: AGGTCCCTTACTTTGCTCTTACC RP-1082: CTCGYYGCAACCCAACTG	*Mnl*I	Edwards-Smith et al., 1999
IL12B-3'UTR A/C	PCR-RFLP	FP1: ATTTGGAGGAAAAGTGGAAGA FP2: AATTTCATGTCCTTAGCCATA	*Taq*I	Davoodi-Semiromi et al., 2002
*IL12B*pro	Allele-specific PCR	FP: GTCAATGGGCATTTGGCTCATATTACC RP1: ATTGGTCCTTCTGTTTTGTCCTAATG TGGGGGCCACATTAGAG RP2: TCTAATGTGGGGGCCACAGC		Mueller et al., 2004

PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism.

Frequencies of alleles and genotypes were calculated by direct counting. Observed frequencies were evaluated for conformity to Hardy-Weinberg equilibrium using the Pearson chi-square test. A chi-square or Fisher's exact test was used to compare the genotype and allele frequencies between ethnic groups. A 2-sided P value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics, version 21 (IBM Corporation, Armonk, NY, USA) and GraphPad Prims 5.01 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

In this study, we examined the DNA samples of 347 individuals to determine the frequencies of gene polymorphisms in the IL-10 and IL-12B gene loci. Sixty-two percent of the study population was male. The ethnic composition of the study population paralleled that of the total Malaysian population, most of whom are Malay (53.9%), followed by Chinese (20.8%), Indian (14.6%), and other races (10.7%).

The distributions of all alleles and genotypes were consistent with Hardy-Weinberg equilibrium. The frequencies of alleles and genotypes for the corresponding cytokine genes among the study population are summarized in Table 2. The *IL-12B* pro1 (CTCTAA) and pro2 (GC) alleles of *IL-12B*pro were equally distributed among our study population. The pro1/pro2 heterozygote was the predominant genotype (52.7%), followed by homozygote pro2/pro2 (28.5%) and pro1/pro1 (18.7%). The *IL-12B* 3'UTR A allele appeared at higher frequency than the C allele; the AC genotype was the predominant genotype (52.2%) (Table 2). *IL-10* promoter haplotypes were characterized based on 3 bi-allelic SNPs. Three haplotypes were detected in our population: ATA, ACC, and GCC. Non-GCC haplotypes (ATA/ATA, ATA/ACC, or ACC/ACC) were frequently (80%) distributed in the population, while the GCC haplotypes (ATA/GCC, ACC/GCC, or GCC/GCC) were relatively rare (20%).

Our results showed that all major ethnic groups in Malaysia shared similar frequencies of *IL-12B* and *IL-10* -592 alleles and genotypes (Table 2). For the *IL-10* -1082 gene locus, however, the G allele was present in only 2.1% of the Chinese subjects in comparison to 12.2% of the Malay (P = 0.0002) and 15.3% of Indian (P = 0.0002) subjects (Table 2). No

differences between females and males or between the patients and healthy volunteers (data not shown) were observed for all allele and genotype frequencies.

Table 2. Frequency of *IL-10* and *IL-12B* gene polymorphisms among the Malaysian population.

Gene		Total [N (%)]	Malay [N (%)]	Chinese [N (%)]	Indian [N (%)]
IL12B					
Allele	*IL12B* pro1	313 (45.1)	162 (44.8)	59 (42.1)	50 (51.0)
	IL12B pro2	381 (54.9)	200 (55.2)	81 (57.9)	48 (49.0)
Genotype	*IL12B* pro1/pro1	65 (18.7)	37 (20.4)	8 (11.4)	12 (24.5)
	IL12B pro1/pro2	183 (52.7)	88 (48.6)	43 (61.4)	26 (53.1)
	IL12B pro2/pro2	99 (28.5)	56 (30.9)	19 (27.1)	11 (22.4)
IL12B-3'UTR					
Allele	A	413 (59.5)	214 (59.1)	82 (58.6)	63 (64.3)
	C	281 (40.5)	148 (40.9)	58 (41.4)	35 (35.7)
Genotype	AA	116 (33.4)	62 (32.3)	21 (30.0)	19 (38.8)
	AC	181 (52.2)	90 (49.7)	40 (57.1)	25 (51.0)
	CC	50 (14.4)	29 (16.0)	9 (12.9)	5 (10.2)
IL10 -1082					
Allele	G	71 (10.2)	44 (12.2)	3 (2.1)[a]	15 (15.3)
	A	623 (89.8)	318 (87.8)	137 (97.9)	83 (84.7)
Genotype	GG	2 (0.6)	1 (0.6)	0 (0.0)	1 (2.0)
	AG	67 (19.3)	42 (23.2)	3 (4.3)[b]	13 (26.5)
	AA	278 (80.1)	138 (76.2)	67 (95.7)	35 (71.4)
IL10 -819					
Allele	C	234 (33.7)	126 (34.8)	39 (27.9)	42 (42.9)
	T	460 (66.3)	236 (65.2)	101 (72.1)	56 (57.1)
Genotype	CC	47 (13.5)	28 (15.5)	7 (10.0)	7 (14.3)
	CT	140 (40.3)	70 (38.7)	25 (35.7)	28 (57.1)
	TT	160 (46.1)	83 (45.9)	38 (54.3)	14 (28.6)
IL10 -592					
Allele	C	234 (33.7)	126 (34.8)	39 (27.9)	42 (42.9)
	A	460 (66.3)	236 (65.2)	101 (72.1)	56 (57.1)
Genotype	CC	47 (13.5)	28 (15.5)	7 (10.0)	7 (14.3)
	CA	140 (40.3)	70 (38.7)	25 (35.7)	28 (57.1)
	AA	160 (46.1)	83 (45.9)	38 (54.3)	14 (28.6)
IL10 haplotypes	Non-GCC (ATA/ATA, ACC/ACC, ATA/ACC)	278 (80.1)	138 (76.2)	67 (95.7)	35 (71.4)
	Heterozygous (ATA/GCC, ACC/GCC)	67 (19.3)	42 (23.2)	3 (4.3)	13 (26.5)
	Homozygous GCC	2 (0.6)	1 (0.6)	0 (0.0)	1 (2.0)

[a]Decreased in Chinese in comparison to Malay (P = 0.0002, OR = 0.16, 95%CI = 0.05-0.52). Decreased in Chinese in comparison to Indian (P = 0.0002, OR = 0.12, 95%CI = 0.03-0.43). [b]Decreased in Chinese in comparison to Malay (P = 0.0002, OR = 0.15, 95%CI = 0.04-0.50). Decreased in Chinese in comparison to Indian (P = 0.0007, OR = 0.12, 95%CI = 0.03-0.46). OR = odds ratio; CI = confidence intervals.

DISCUSSION

We determined the distribution of *IL-10* and *IL-12* cytokine gene polymorphisms among the multiethnic Malaysian population. The *IL-12B* pro allele distribution in the Malaysian population was generally similar to that reported for populations in Thailand, Australia, and Germany (Morahan et al., 2002; Mueller et al., 2004; Naka et al., 2009). The *IL-12B* 3'UTR A/C allele frequencies of Malaysians were consistent with those reported for Thai, Chinese, and Japanese populations (Sodsai et al., 2011). However, the frequency varied when compared to that of Taiwanese, Koreans, Caucasians, Africans, and Italians (Trejaut et al.,

2004; Sodsai et al., 2011). Our findings, together with those of previous studies, support variations in the frequency of specific gene polymorphisms depending on the ethnic composition.

We examined the frequencies of *IL-12B* and *IL-10* promoter gene polymorphisms among multiethnic Malaysians. The regulatory balance between IL-12 and IL-10 has been shown to influence the development of appropriate Th phenotypes. Inheritance of specific gene variants may alter the direction of immune responses and affect the pathogenesis of immune-mediated diseases. The associations between *IL-12B* gene variants and diseases such as type 1 diabetes (Morahan et al., 2001), asthma (Morahan et al., 2002), malaria (Marquet et al., 2008; Naka et al., 2009), and hepatitis C (Mueller et al., 2004; Hegazy et al., 2008) are well documented. Most gene association studies, however, were reported for Caucasian populations. Thus, the findings may not be reflective of the Asian or African populations because of the population-specific genetic variations. Additional population-based gene association studies should be conducted, as these studies are currently lacking for Malaysians. Our findings may be useful for future studies of genetic association with risks for diseases among the Malaysian population.

The presence of the *IL-10* promoter -1082G allele or GCC haplotype is associated with an increased level of IL-10 production (Turner et al., 1997). Our results suggest that most of the Malaysian population has high frequencies of alleles and haplotypes associated with low IL-10 production. The high IL-10 producer -1082G allele was detected at a low frequency (10%) and was similar to that in Koreans (13%) (Yazici et al., 2009). In contrast, the -1082G allele was distributed more frequently among Caucasians (36%) (Yazici et al., 2009), Mexicans (36%) (Meenagh et al., 2002), Italians (39%) (Trejaut et al., 2004), Greeks (38%) (Trejaut et al., 2004), African-Americans (32%) (Trejaut et al., 2004), and the Dutch (49%) (Skorpil et al., 2007). *IL-10* promoter gene polymorphisms may influence the susceptibility to and outcome of viral infection (Helminen et al., 1999; Shrestha et al., 2007; Zhang et al., 2010; Sun et al., 2013). Low IL-10-producing genotypes have been associated with a greater risk for diseases such as inflammatory bowel disease (Tagore et al., 1999), asthma, systemic lupus erythematosus (Hee et al., 2008), and arthritis (Fife et al., 2006; Hee et al., 2007). In our study, the Malaysian Chinese population showed the lowest frequency of the 1082G allele (2%), which is consistent with a previous report in the Chinese populations of Singapore, China, and Taiwan (Trejaut et al., 2004). Genetic variation between ethnic groups may explain the ethnic differences in specific disease prevalence and disease profiles (Wang et al., 1997; Hee et al., 2008; Bhoo-Pathy et al., 2012; Lu and Nordin, 2013). In Malaysia, for instance, systemic lupus erythematosus is most prevalent amongst the Chinese ethnic group compared to Malays and Indians (Hee et al., 2008). The low GCC haplotype frequency among systemic lupus erythematosus patients (Hee et al., 2008) may be associated with the low frequency of the GCC haplotype observed in general among the ethnic Chinese population. These observations may explain the higher susceptibility of ethnic Chinese to systemic lupus erythematosus. Therefore, gene variations in the different ethnic groups should be further analyzed for clinical relevance.

In conclusion, we report here the allele and genotype frequencies of *IL-10* and *IL-12B* gene polymorphisms among the multiethnic Malaysian population. The genetic variants between the ethnic groups of Malaysia suggest the importance of understanding ethnic variation in genetic susceptibility to diseases and in response to infections. Overall, our findings were consistent with those reported for the Asian population for the *IL-12B* and *IL-10* genes, but markedly different from those for the African and Caucasian populations.

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